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Thank you.

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Original Article

A Histiocyte-Specific Marker in the Diagnosis of Malignant Fibrous Histiocytoma

Use of Monoclonal Antibody KP-1 (CD68)

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KP-1 (CD68) is a recently described monoclonal antibody to a cytoplasmic epitope present on tissue histiocytes and macrophages. To determine the specificity and sensitivity of this marker in the evaluation of cases of malignant fibrous histiocytoma (MFH), this reagent and a panel of commercially antibodies were used to stain formalin-fixed paraffin sections from 25 cases of MFH and 25 other tumors, including a variety of soft-tissue sarcomas. Eighteen of 25 cases of MFH stained for KP-1 (72%), whereas all other tumors were negative, including 12 cases of pleomorphic soft-tissue sarcoma other than MFH. The percentage of tumor cells staining for KP-1 varied. In 11 cases KP-1 was only focally present, but staining was of a high intensity and associated with minimal nonspecific or background staining. Pleomorphic histiocytic cells and spindle cells from storiform tumors were strongly decorated with antibodies to KP-1 in most cases, and antigen also was present on tumor giant cells. Although alpha-1-antitrypsin and alpha-1-chymotrypsin stained a higher percentage of cases of MFH (92%), immunoreactivity for these markers also was noted in other tumors. Because of its specificity as a histiocyte marker, KP-1 is a useful component in a panel of antibodies for the characterization of soft-tissue sarcomas and the diagnosis of MFH. (Key words: KP-1; CD68; Malignant fibrous histiocytoma; Immunoperoxidase) Am J Clin Pathol 1992; 97:759-763

Malignant fibrous histiocytoma (MFH) is one of the most common soft-tissue tumors of the adult.[1] More common in the elderly, the most frequent subtype is the storiform-pleomorphic variety.[1] The cell of origin for this tumor is not always known and the tumor frequently poses a problem in differential diagnosis. Despite its characteristic mixture of storiform and pleomorphic features, this subtype can easily be confused with a variety of pleomorphic soft-tissue tumors, including pleomorphic liposarcoma, pleomorphic rhabdomyosarcoma, malignant schwannoma, and leiomyosarcoma. Electron microscopy has had a mainly confirmatory role in the diagnosis because there are no specific ultrastructural features, and many tumors contain fibroblasts, histiocytes, and myofibroblasts similar to those present in MFH.[2,3] As a result, there have been many attempts to apply immunohistochemical stains to cases of MFH to characterize these tumors and distinguish them from other pleomorphic high-grade sarcomas.[4-16] Initially immunohistochemical stains for lysozyme, alpha-1-antitrypsin (AAT) and alpha-1-chymotrypsin (ACT)[5,8,10,11,16] were suggested as specific markers for MFH. Subsequent studies, however, indicated that these markers limited sensitivity and specificity for the diagnosis.[15,17] More recent series have stressed the complexity and unreliability of intermediate filament expression as markers for sarcomas, and even cytokeratin- and neurofilament-positive cases of MFH have been reported.[9,13,18,19] Frustration with the currently available alternatives for the immunohistochemical characterization of MFH led us to evaluate an antibody, KP-1. This monoclonal antibody was developed against a lysosomal fraction of human lung macrophages and has a broad reactivity against cells of mononuclear phagocytic lineage in routinely processed formalin-fixed tissue sections.[20] We evaluated 25 cases of MFH and a variety of other tumors, including pleomorphic sarcomas to identify a more spe-

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TABLE 1. PANEL OF ANTIBODIES USED TO CHARACTERIZE SOFT-TISSUE SARCOMAS

Antibody	*Type of Antibody*	*Source*	*Catalogue #*	*Dilution*
Alpha-1 antitrypsin	Rabbit polyclonal	DAKO	A012	1/700
Alpha-1 antichymotrypsin	Rabbit polyclonal	DAKO	A022	1/1,500
Lysozyme	Rabbit polyclonal	DAKO	A099	1/250
S-100	Rabbit polyclonal	DAKO	Z311	1/300
Myoglobin	Rabbit polyclonal	DAKO	A324	1/1500
KP-1 (CD 68)	Mouse monoclonal	DAKO and D.Y. Mason, Oxford, UK	M814	1/400
Keratin AE1/AE3	Mouse monoclonal	Boehringer-Mannheim	#1124 161	1/50
Actin	Mouse monoclonal	Enzo	MA-931	1/40,000
Desmin	Mouse monoclonal	DAKO	M760	1/20
Factor VIII (F8)	Mouse monoclonal	DAKO	M616	1/30
Vimentin	Mouse monoclonal	DAKO	M725	1/30

cific and sensitive marker for MFH. Staining for KP-1 also has implications concerning the role of cells from the monocyte/macrophage system in the histogenesis of these neoplasms.

MATERIALS AND METHODS

Twenty-five cases of malignant fibrous histiocytoma and 25 cases of a variety of other tumors were collected from the files of the Surgical Pathology Department of UCLA Medical Center and Cedars Sinai Medical Center. The diagnosis of MFH was made according to criteria of Enzinger and Weiss[1] and confirmed by at least two independent pathologists.

Sections from formalin-fixed, paraffin-embedded tissue blocks were mounted on poly-l-lysine–coated slides and immunostaining was performed using a panel of commercially available antibodies (Table 1) using techniques previously described.[21]

Sections were incubated for 15 minutes with 3% methanolic peroxide to consume endogenous peroxidase. Slides stained for KP-1 were pretreated with trypsin (0.7 mg/mL phosphate-buffered saline at 37 °C for 30 minutes). Sections were then incubated with monoclonal antibodies to KP-1 (supplied by Dr. D. Y. Mason, Oxford, UK and commercial antibody KP-1 (CD68) from Dakopatts, Inc., Santa Barbara, CA) at dilutions of 1:50 to 1:400 for 1 hour, followed sequentially by peroxidase-conjugated rabbit anti-mouse and swine anti-rabbit antibodies (Dakopatts) diluted 1:100 in phosphate-buffered saline with 1% normal swine serum and 1% human AB serum. In the case of antibodies produced in rabbits, staining was performed with primary anti-serum, swine anti-rabbit immunoglobulins and rabbit peroxidase anti-peroxidase soluble complexes, as previously described.[22] In all cases, antibody localization was performed using the peroxidase reaction with 3.3′-diaminobenzidine tetrahydrochloride (Aldrich Chemical Co., Milwaukee, WI) as the chromogen. Slides were counterstained with hematoxylin and mounted with Permount.

Identical staining resulted with the antibody obtained from Dr. Mason and the commercial antibody. Antibody was tested at dilutions 1:50, 1:100, 1:200, 1:400, and 1,000. Dilution at 1:400 was selected for most cases because this gave strong positive staining with minimal background.

RESULTS

Clinical Findings

Seventeen cases of MFH occurred in men and the rest in women. Twelve occurred in the lower extremity, seven in the upper extremity, five in the retroperitoneum, and one in the head and neck region. Ages of the female patients ranged for 62 to 82 years (mean, 73.4 years) and ages of the male patients ranged from 48 to 80 years (mean, 65 years).

TABLE 2. IMMUNOHISTOCHEMICAL STAINING PATTERN FOR MFHs

Tumor Type	*KP-1*	*AAT*	*ACT*	*LYS*	*S100*	*ACTIN*	*DES*	*MYO*	*F8*	*KER*
Pleomorphic/storiform (n = 15)	12	11	14	2	1	3	1	0	0	1
Myxoid (n = 5)	2	3	4	0	0	3	0	0	0	0
Inflammatory (n = 3)	3	3	3	1	1	2	1	0	0	1
Giant cell (n = 2)	1	2	2	1	0	0	0	0	0	1

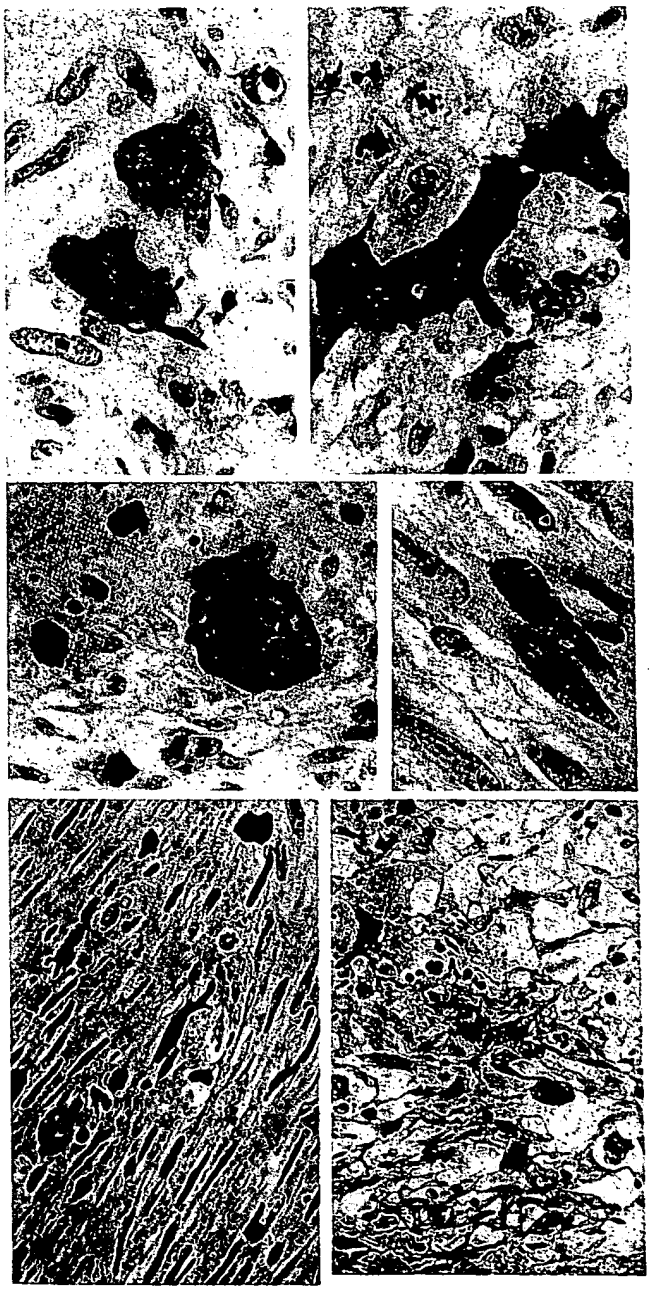
AAT = alpha-1 antitrypsin; ACT = alpha-1 antichymotrypsin; LYS = lysozyme; DES = desmin; MYO = myoglobin; F8 = Factor VIII; KER = keratin.

Gross Pathologic Findings

Tumors were large (ranging from 3 cm to 18 cm; mean, 10.5 cm), firm fleshy, gray-tan masses. Many contained extensive areas of necrosis and most were situated in the deep soft tissues or retroperitoneum, often infiltrating skeletal muscle and eroding bone.

Light Microscopic Findings

Fifteen cases of MFH were classified as the storiform-pleomorphic subtype, five were predominantly myxoid tumors, three were characterized as the inflammatory subtype, and two were of the giant cell variety. No case of angiomatoid type was studied. The tumors were often heterogeneous, showing foci characteristic of two or more histologic subtypes. Such cases were classified according to the predominant histologic subtype. All of the cases selected had been sampled extensively and the most representative area was chosen for immunostaining. One case was considered a postirradiation sarcoma, occurring within the radiation field 11 years after treatment for Hodgkin's disease.



Immunohistochemical Staining

The results of immunostaining for KP-1 and a panel of other antibodies are shown in Table 2. Staining was graded + (focal), ++ (moderate), or +++ (majority) based on the percentage of cells that stained with the corresponding antibody. A grade of + represents staining of 25% or less of the tumor cells, ++ indicates staining of 25% to 50% of the tumor cells, and +++ indicates that more than 50% of the tumor cells stained.

Eighteen of 25 cases (72%) of MFH stained for KP-1. In 11 cases staining was focal (+), in 6 staining was ++, and in 1 the majority of tumor cells were positive +++. In all cases intensity of staining was strong with the cytoplasm of the neoplastic cells. In addition to diffuse cytoplasmic staining, many cells exhibited "block and dot" patterns of intracytoplasmic staining (Fig. 1). Staining was noted in bizarre multinucleated histiocytic cells and blander cells in storiform areas (Figs. 2 and 3). Although two or five myxoid tumors stained for KP-1, and one case showed staining of most tumor cells (Fig. 4), this subtype had the smallest percentage of cells that expressed the antigen. Twelve of fifteen (80%) of the cases of storiform/pleomorphic MFH were at least focally positive for KP-1. The one giant cell variant of MFH showed strong staining of almost all tumor cells, including bizarre multinucleate forms. KP-1 did not stain accompanying granulocytes so that interpretation of staining of the inflammatory subtype of MFH was not hampered. Even in tumors with extensive necrosis there was minimal background staining.

FIG. 1 (*upper*). MFH with dot (left) and block (right) pattern of staining for KP-1 (hematoxylin counterstain, ×500).

FIG. 2 (*middle*). Strong diffuse cytoplasmic staining for KP-1 (CD68) in giant (left) and spindle cells (right) from a typical malignant fibrous histiocytoma (hematoxylin counterstain, ×500).

FIG. 3 (*lower left*). Spindle cell pattern in MFH with strong cytoplasmic staining for KP-1 (hematoxylin counterstain, ×500).

FIG. 4 (*lower right*). Myxoid MFH with large malignant cells staining strongly for KP-1 (hematoxylin counterstain, ×500).

TABLE 3. IMMUNOHISTOCHEMICAL STAINING PATTERN FOR NON-MFH SARCOMAS

Tumor Type	*KP-1*	*KER*	*S-100*	*AAT*	*ACT*	*ACTIN*	*DES*	*Others*
Neurogenic sarcoma (n = 2)	0	0	2	0	0	0	0	Leu 7
Leiomyosarcoma (n = 2)	0	0	0	1	1	2	2	
Liposarcoma (n = 2)	0	0	2	0	2	0	0	
Rhabdomyosarcoma (n = 2)	0	0	0	1	—	2	2	Myoglobin
Fibrosarcoma (n = 2)	0	0	0	0	0	0	0	
Angiosarcoma (n = 1)	0	0	0	1	1	—	—	Factor VIII

See Table 2 for definitions of abbreviations.

Sensitivity of staining of MFHs for KP-1 was 72%, compared with 76% for AAT and 96% for ACT. Specificity of staining for KP-1 was 100% (no false-positive results), however, compared with 73% for AAT and only 66% for ACT.

Results of staining of cases of MFH for other tissue antigens conformed closely to patterns reported in the literature. Positive staining with AAT and ACT was observed in 76% and 92% of tumors, respectively. Although ACT appeared more sensitive in staining cases of MFH, this was often accompanied by nonspecific background staining. Only 4 cases stained with antibody to lysozyme (16%). All 25 tumors stained strongly for vimentin, and 2 cases revealed focal staining of tumor cells for S100 protein. Three cases stained focally with antibodies to cytokeratins. Two cases stained positively for desmin, and 9 of 25 cases (36%) stained for actin with variable intensity. None of the MFH tumor cells stained with antibodies to myoglobin or Factor VIII.

Results of staining for KP-1 in non-MFH tumors are shown in Tables 3 and 4. These included 13 cases of sarcoma other than MFH, 3 cases of malignant melanoma, 3 adenocarcinomas of the lung, breast and colon, 2 malignant lymphomas, 2 carcinoid tumors, 1 astrocytoma, 1 transitional cell carcinoma of the bladder, and 1 pleural mesothelioma, all of which were negative for KP-1. Reactive histiocytes associated with the host response stained strongly but did not interfere with interpretation of results.

DISCUSSION

KP-1 is a monoclonal antibody that recognizes an antigen most likely to be present on membrane-bound lysosomes of cells derived from the monocyte/macrophage system.[20] This antigen is resistant to formalin and B5 fixation and is present on a variety of tissue macrophages, including Kupffer cells, germinal center macrophages, alveolar macrophages, and osteoclasts of bone.[20] Pulford and associates[20] suggested that anti–KP-1 antibodies may be useful in the diagnosis of "malignant histiocytic tumors" but did not evaluate soft-tissue neoplasms of presumed fibrohistiocytic origin. The availability of a unique monoclonal antibody specific for the monocyte–macrophage system that retains reactivity in fixed tissue sections offers a new approach to the characterization of MFH. None of the immunohistochemical markers that have been evaluated previously are cell lineage specific, and results of immunostaining of these tumors have been problematic.

Ultrastructural and immunohistochemical studies indicate that MFHs originate from a primitive mesenchymal cell that may show divergent lines of differentiation, expressing fibroblastic, histiocytic, and even myofibroblastic phenotypes.[1,2,6,23] In our series, more than 70% of cases of MFH stained for KP-1, confirming histiocytic differentiation for most of these tumors. In cases of MFH, staining was not confined to cells with histiocytic appearance on histologic sections but also was present in areas with storiform, myxoid, and giant cell patterns. Evidence that all cell types in MFH (immature, histiocyte-like, fibroblast-like, and giant cells) may be derived from differentiated histiocytes is supported by studies in nude mice that developed pleomorphic MFHs when injected with neoplastic histiocyte clones.[3]

Staining for KP-1 could not be predicted from evaluation of the hematoxylin-and-eosin–stained sections. Whether the unstained tumors represent dedifferentiated histiocytes, primitive mesenchymal cells not staining for KP-1, or may even be derived from other cell types remains to be elucidated. The fact that KP-1 stained many tumors only focally supports the contention that most MFHs originate from primitive totipotential mesenchymal cells, some of which express the KP-1 antigen, indicating focal histiocytic differentiation. True histiocytic differentiation in MFHs is, however, supported by Strauchen and Dimitriu-Bona,[18] who found monocyte–macrophage markers (T-200, Ia, MoS-1, MoS-39, MoR-17) on both spindle and histiocyte-like MFH tumor cells. Studies in syngeneic mice that developed MFH-like tumors after inoculation with bone marrow macrophages also suggest that these tumors may be derived from histiocytes.[24]

The value of KP-1 as a marker for MFH lies in its specificity for cells of the mononuclear phagocytic system. Alpha–1-antitrypsin and alpha–1-chymotrypsin appear to

TABLE 4. IMMUNOHISTOCHEMICAL STAINING PATTERN FOR OTHER NON-MFH TUMORS

Tumor Type	*KP-1*	*KER*	*S-100*	*Others*
Melanoma (n = 3)	0	0	3	HMB 45, AAT one case
Adenoca (n = 3)	0	3	0	EMA
Lymphoma (n = 2)	0	0	0	LCA, B-cell markers
Carcinoid (n = 2)	0	1	0	Chromogranin, synaptophysin
Mesothelioma (n = 2)	0	2	0	
Urothelial ca (n = 1)	0	1	—	
Astrocytoma (n = 1)	0	0	—	Glial fibrillary acidic protein

See Table 2 for definitions of abbreviations.

be more sensitive markers because a greater percentage of cases of MFH were stained, but they suffer from a lack of specificity with staining of a variety of tissues and tumors, including other soft-tissue neoplasms.[17] None of the 25 tumors other than MFH in this series stained for KP-1, an encouraging preliminary result. Because KP-1 may only be present focally in cases of MFH, we recommend its inclusion in a panel of antibodies for the diagnosis of soft-tissue sarcomas.

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